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BIOCHEMICAL CHARACTERIZATION OF NEONATAL MULTIPLE SULFATASE DEFICIENT (MSD) DISORDER CULTURED SKIN FIBROBLASTS

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SUMMARY: Cultured skin fibroblasts of a patient with a neonatal onset of MSD can be distinguished from usual type of MSD cells by enzyme assays of arylsulfatases A, B and C activities, effect of thiosulfate on arylsulfatase A activity and $^{35}SO_4$-acid mucopolysaccharide accumulation and degradation. These data suggest that neonatal onset of MSD is a distinct disorder from usual type of MSD in term of genetic occurence.

Multiple sulfatase deficiency (MSD) is clinically characterized by gargoyl like face, bony changes, hepatosplenomegaly, ichthyosis and neurological symptoms. The clinical symptoms in MSD first appear between the ages of 12 and 18 months. Biochemically, this disorder is unique, since more than seven different sulfatase activities are deficient or markedly reduced in patients' tissues, leukocytes and cultured skin fibroblasts (1-5). Furthermore, in MSD patient tissues micellaneous sulfated compounds such as sulfatide, cholesterol sulfate and acid mucopolysaccharides (AMPS) have been accumulated (1-5).

Recently, Vamos et al. (6, 7) reported that in neonatal onset of MSD, clinical features were much more involved than those of the usual type of MSD. The activities of arylsulfatases A, B and C in the patient's cultured skin fibroblasts were greatly reduced. This communication characterizes the biochemical features of neonatal MSD and distinguishes this disorder from typical MSD.

MATERIALS AND METHODS:

Cell cultures: Skin fibroblasts were obtained from a case of neonatal MSD and four typical cases of MSD (one from our case (8), one from Prof. H.

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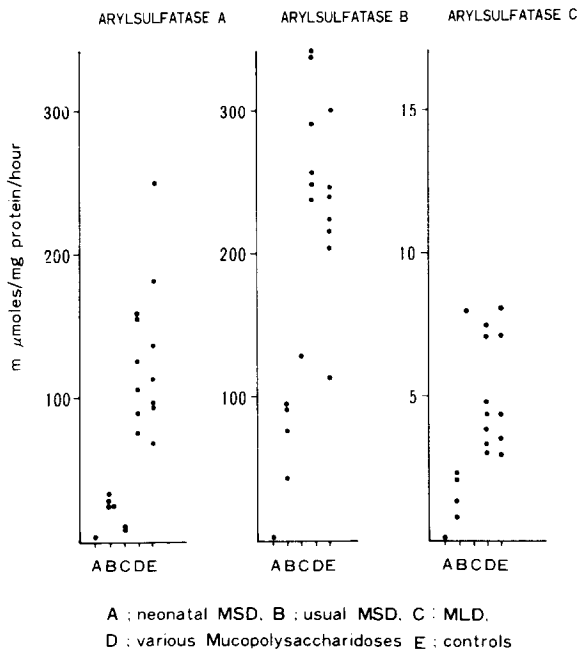

Fig. 1. Various sulfatase activities in cultured skin fibroblasts from neonatal and usual type of MSD.

Kihara and two from New Jersey Cell Repository (GM 3245 and GM 2704). The culture were grown and maintained by standard techniques. The cells were grown in Ham's F-10 nutrient mixture (Gibco, Grand Island, N.Y.USA) supplemented with 10% fetal calf serum in an atmosphere of 5 % CO_2 in 75 cm^2 Falcon tissue culture flaks.

Enzyme assays: Arylsulfatases A and B activities were determined using p-nitrocatechol sulfate as a substrate according to Baum et al. (9). Arylsulfatase C activity was measured using 4-methylumbelliferyl sulfate as described previously (4).

^{35}S-AMPS accumulation and degradation in cell cultures: Intracellular accumulation and degradation of $^{35}SO_4$-AMPS was essentailly carried out as described by Fratantoni et al. (10). Duplicate cell cultures were grown in the presence of $^{35}SO_4$-sodium sulfate (1 uCi/ml medium) for 3 and 7 days. The medium was then discarded and the cultures were washed twice with 0.9 % saline. Fresh medium was added and a chase of labeled compound followed for 48 hour. Intracellular radioactivity was measured as described previously (4).

Effect of thiosulfate on arylsulfatases A and B: The effect of thiosulfate on arylsulfatase A activity in cultured skin fibroblasts of patients with MSD was carried out as described by Kresse et al. (11). Duplicate cultures were grown in the presence of 2 mM thiosulfate for 7 and 14 days, and measured the activities of arylsulfatases A and B.

RESULTS:

Various sulfatase activities in cultured skin fibroblasts: Fig. 1. shows arylsulfatases A, B and C activities in cultured skin fibroblasts from a patient with neonatal MSD, compared with those of typical MSD.

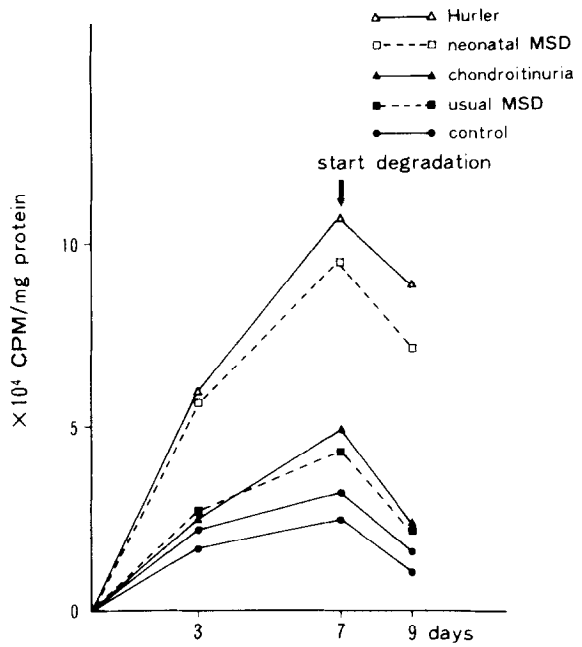

Fig. 2. $^{35}SO_4$-acid mucopolysaccharide accumulation and degradation in neonatal and usual type of MSD. The method used was carried out as described in the text.

The activities of these enzymes in neonatal MSD cells are practically absent, whereas those activities from the usual type of MSD cells were significantly higher; arylsulfatase A activity from four different cell lines of the usual type of MSD was 10-15% of control values arylsulfatase B activity was almost 30-40% of control values and arylsulfatase C activity was 20-30% of control value.

Intracellular accumulation and degradation of $^{35}SO_4$-AMPS: Intracellular $^{35}SO_4$-AMPS accumulated by fibroblasts from Hurler, neonatal type of MSD, usual type of MSD, chondroitin sulfate uria and control is shown in Fig. 2. Intracellular accumulation of $^{35}SO_4$-AMPS in neonatal MSD cells was three to four times higher than that of controls and twice that of typical MSD. Intracellular degradation of $^{35}SO_4$-AMPS in neonatal MSD after a 48 hour chasing was about 25% of initial rate whereas typical MSD cells degraded about 50% of intracellular AMPS after a 48 hour chasing.

Effect of thiosulfate on arylsulfatase A activity: Table 1 shows the effect of thiosulfate on arylsulfatases A and B activities in neonatal

Table 1. Effect of thiosulfate (2 mM sodium thiosulfate) on arylsulfatases A and B activities in neonatal and usual MSD fibroblasts.

	Arylsulfatase	
	A	B
	(mμ moles / mg protein / h)	
Usual MSD		
0 day	17.1	81.3
	21.9	65.9
8 days	36.4	33.3
14 days	67.5	135.0
	30.9	77.4
Neonatal MSD		
0 day	8.8	35.2
	11.9	30.0
8 days	10.7	30.7
14 days	9.3	23.4
	15.6	39.0

and the usual type of MSD fibroblasts. The cells from the usual type of MSD could induce arylsulfatase A activity after 14 days of culturing in the presence of 2 mM thiosulfate, whereas neonatal MSD cells could not induce the activity of arylsulfatase A in the same condition. The activity of arylsulfatase B in both the usual and neonatal type of MSD cells was not significantly induced in the presence of 2 mM thiosulfate.

DISCUSSION:

A case of neonatal MSD was clinically more involved than those of the usual type of MSD. Severe bony changes, grotesque face, CNS involvement, hydrocephalus, hepatosplenomegaly, corneal clouding and ichthyosis were present. The patient also excreted a large amount of AMPS in urine. These clinical features suggest that a neonatal onset of MSD is a biochemically different disorder from the usual type of MSD. In order to characterize neonatal MSD, we performed three kinds of experiments; (1) analysis of enzyme activities of arylsulfatases A, B and C (2) analysis of accumulation and degradation of $^{35}SO_4$-AMPS in both types of MSD cells, and (3) a test of

the effect of thiosulfate on arylsulfatase A induction. First, arylsulfatases A, B and C in neonatal MSD cells could be distinguished from the usual type of MSD contained small residual enzyme activities of arylsulfatases A, B and C. The relatively higher residual activity of several sulfatases in cultured skin fibroblasts are consitent findings (4,5). In the cultured condition of F-10-CO_2 atmosphere, these enzyme activities were not induced and remained constant, though Fluharty et al. (12) reported that in the cultured condition with HEPES-MEM arylsulfatase A activity in usual MSD cells was restored. Arylsulfatase A activity in neonatal type of MSD cells was not changed by the condition of cultured medium (unpublished observation). The incorporation of $^{35}SO_4$-sodium sulfate into AMPS in neonatal type of MSD showed significantly higher accumulation than either those of typical MSD or control cells. These findings confirmed that de Novo activity of arylsulfatase B in neonatal type of MSD cells was much lower than that of the usual type of MSD. The major accumulated radioactive compound in both neonatal and usual type of MSD was dermatan sulfate and there was no difference on radioactivity into each AMPS between two types of MSD cells (unpublished observation). The effect of thiosulfate on arylsulfatase A in the usual type of MSD cells shows that arylsulfatase A activity was increased about 2 to 3 times by the presence of 2 mM thiosulfate. However, the activity of arylsulfatase A in neonatal type of MSD was not induced by the addition of 2 mM thiosulfate in the medium. This biochemical difference might be due to qunatitative differences in amounts of various sulfatase activities. Furthermore, the failure to complement arylsulfatases A and B activities between neonatal and usual type of MSD cells supports that a genetic defect in both disorders are in the same genom (to be published). Further works are necessary to elucidate a primary defect for multiple sulfatase deficiencies in this disorder.

ACKNOWLEDGEMENTS

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